GENETICS AS A TOOL FOR STUDYING GENE STRUCTURE

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By making use of current ideas about the structure of genes it is possible to develop a formal scheme which will account for the continuity of genic specificity in gene reproduction, and for the initiation of gene-controlled reactions in the cytoplasm. Such a formulation gives a pattern into which many diverse genetic observations can be fitted and suggests definite lines of experimental approach which were not otherwise apparent.

Two characteristics of genes are important to the development of this scheme. On the one hand, genes differ greatly in their specificities as can be seen from the diverse gene-controlled reactions reported by Tatum and Beadle ('45) earlier in this Conference. On the other hand, genes are extraordinarily alike in all other respects, including their ultimate chemical constitution as understood at this time. This characterization of genes reminds one of similar properties of antibodies which resemble one another closely except in their specific relationships to their homologous antigens.

That antibody specificity resides in the unique surface configuration of a particular antibody molecule is strongly suggested by studies on the antigenic relationships of simple chemical substances (Landsteiner, '36; Marrack, '38). Pauling, Campbell and Pressman ('43) have pointed out how the specific surface of an antigen can serve as a template upon which the antibody surface is determined, the surfaces of the two molecules then being mutually complementary in shape and in the arrangement of reactive groups (i. e., oppositely charged groups, groups capable of forming hydrogen bonds, etc.).

Complementary, antigen-antibody like surfaces have been suggested for other biological systems. One of these is the relationship between enzymes and their substrates, such as the relations between specific glucosidases and chemically modified glucosides recently reviewed by Pigman ('44). From the antigen-antibody like reactions between the surface and underlying substances in certain invertebrate eggs, Tyler ('40) has suggested that such complementary structures may be an important feature in the architecture of all cells. On the basis of physical-chemical considerations, Pauling and Delbrück ('40) postulated that most biological syntheses involved the building of complementary surfaces, pointing out that complementary surfaces can be identical under certain circumstances.

While there is no direct evidence indicating that genic specificity resides in the unique surface configuration of the gene, there are several reasons for making that inference. In the first place, the specific active surfaces of enzymes (Tatum and Beadle, '45) and naturally occurring antigens (references in Emerson, '44) are themselves gene controlled. The simplest interpretation would be that surfaces of

the enzymes and antigens are derived from the surfaces of the genes involved. In the second place, genic specificity is transmitted through molecules of different chemical make-up. For example, the nucleoproteins in the chromosomes of the fish change during the ripening of the sperm from nucleo-histones to nucleo-protamines with no break in the continuity of genic specificity. This situation seems to me to be readily understood on the basis of surface configurations, especially when we recall that polysaccharide antigens determine the surfaces of gamma-globulin antibodies.

There are two general routes by which the surface structure of a gene could be transmitted in gene reproduction, and from the gene to the enzyme, or antigen. The gene could reproduce by forming a complementary, antibody-like template upon which the surface of the new gene is synthesized, or the specific surface could be copied directly if the gene has a structure like that proposed by Delbrück ('41; cf. Gulick, '44). The primary gene product in the cytoplasm could obtain its specific surface in either of these ways.

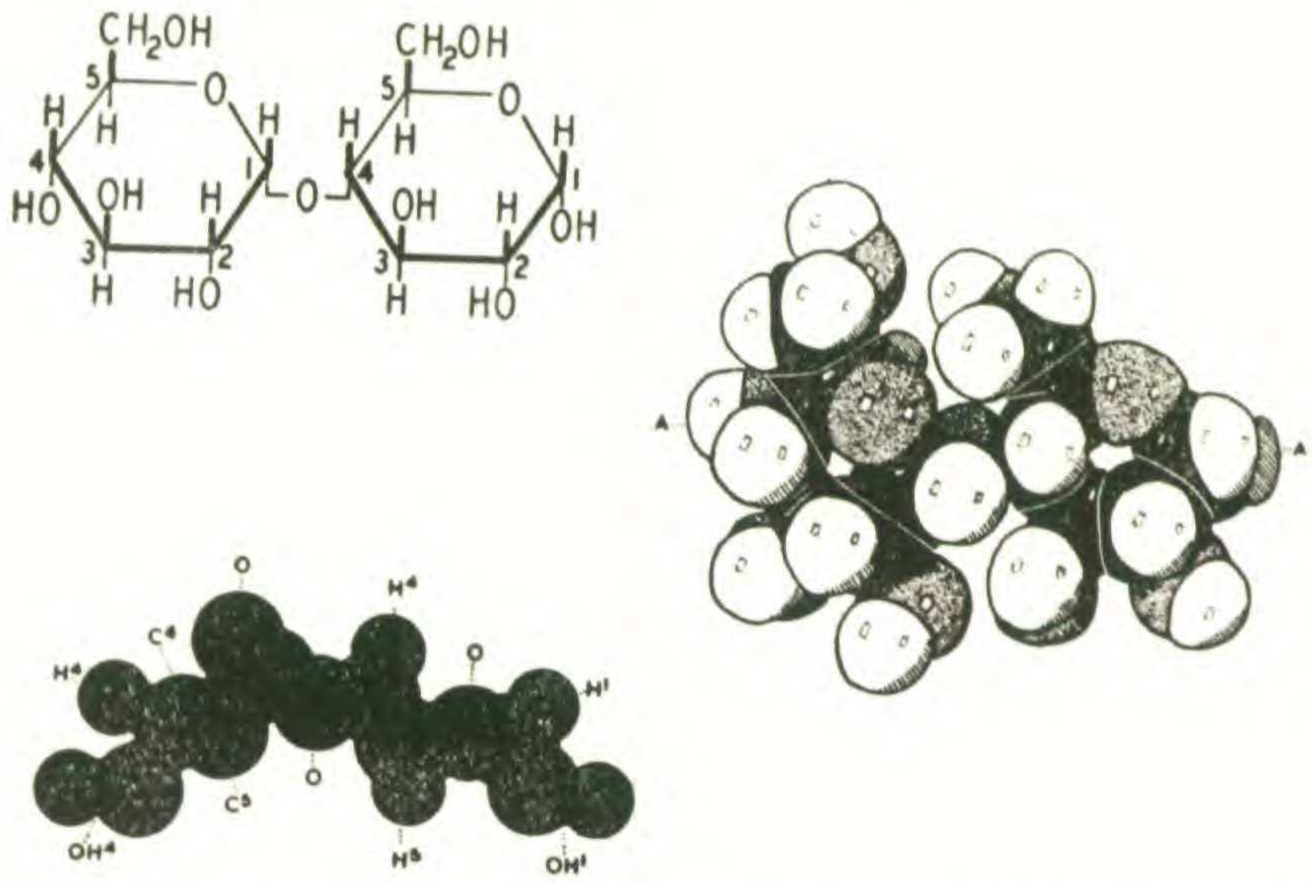

Fig. 1. Maltose molecule; upper left, structural formula; center right, surface view of model in same orientation as formula (black, carbon; stippled, oxygen; others, hydrogen); lower left, section through model, along line A—A.

These routes by which specific surfaces can be transmitted are shown schematically in figs. 1, 2, and 3, in which the gene determining the active surface of the enzyme maltase is used as an example. In the first figure a molecule of maltose is represented in three ways, by the structural formula, by a surface view of a model, and by a section through the model.

From what is known about the relationships between certain groups on the maltose molecule and the specific enzymes, it is possible to guess the surface of the sugar molecule which may be associated with the enzyme surface. Of special importance are the alpha position of the glucoside linkage (differentiating maltose from cellobiose) and the position of the hydroxyl group on carbon atom 4 of the

glucoside residue (which distinguishes glucosides from galactosides). Judging by the specificities of beta-glucosides (Pigman, '44), relatively minor substitutions on carbon atoms 2 and 3 of the glucoside radical would destroy the specificity, whereas minor substitutions on carbon atom 6 of this residue, or fairly substantial substitutions on the other glucose residue, should only lessen the specificity. These considerations make it seem probable that the enzyme attaches either to the upper surface (as illustrated in fig. 1), or to the lower surface. The upper surface has been chosen for purposes of illustration.

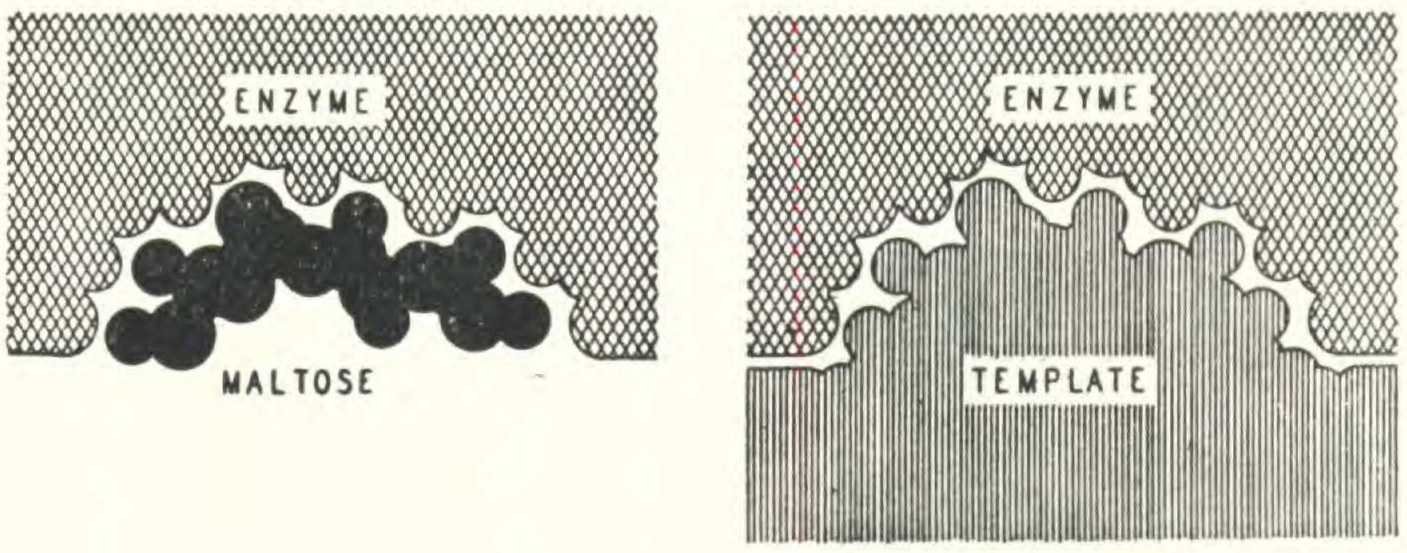

Fig. 2. Complementary surfaces: left, section through maltase (ENZYME) with associated maltose molecule; right, maltase and complementary template.

The diagram at the left in fig. 2 represents a section through a molecule of the enzyme maltase which has a molecule of maltose associated with its specific active surface. The surface drawn for the enzyme is not intended to be exact in any way, but is intended to represent a complementary relationship to the surface of maltose in the same sense as antigen and antibody molecules are complementary. At the right in this figure is a section through the same enzyme and a complementary template upon which the surface configuration of the enzyme might be determined. As drawn, the surface of the template is identical to the corresponding surface of maltose. They are not identical, one being a carbohydrate, the other presumably a nucleoprotein, but they must be similar to the extent that both are complementary to the surface of the enzyme.

Figure 3 is a scheme illustrating the possible routes of gene reproduction and gene action. The specific surface configuration of the gene (G) may be reproduced directly as shown by the dotted line, or indirectly through the intervention of a complementary template (T^G). If both gene and template are part of the genic material it is purely a matter of convenience which is called gene and which template. If the enzyme (E) has a surface configuration identical to that of the gene (upper half of figure) it may obtain this surface through a complementary template (T^E) or directly (dotted line), depending upon the structure and mode of synthesis of the molecules involved. The lower part of the figure shows how the enzyme might have a surface complementary to that of the gene, in which case there could be no surface in the genic material identical to that of the enzyme

unless the gene reproduces by means of a template.

Except for intermediate steps in gene action (which must duplicate the steps illustrated), this diagram exhausts the possible ways by which specific surfaces can be transmitted according to the postulates outlined above. It should be possible to distinguish between these alternative routes experimentally. Sturtevant ('44) and I (Emerson, '44) have pointed out how antibodies to natural antigens could cause mutations, provided the gene and antigen have similar surfaces, and we have interpreted certain examples on that basis. The argument used is briefly this:

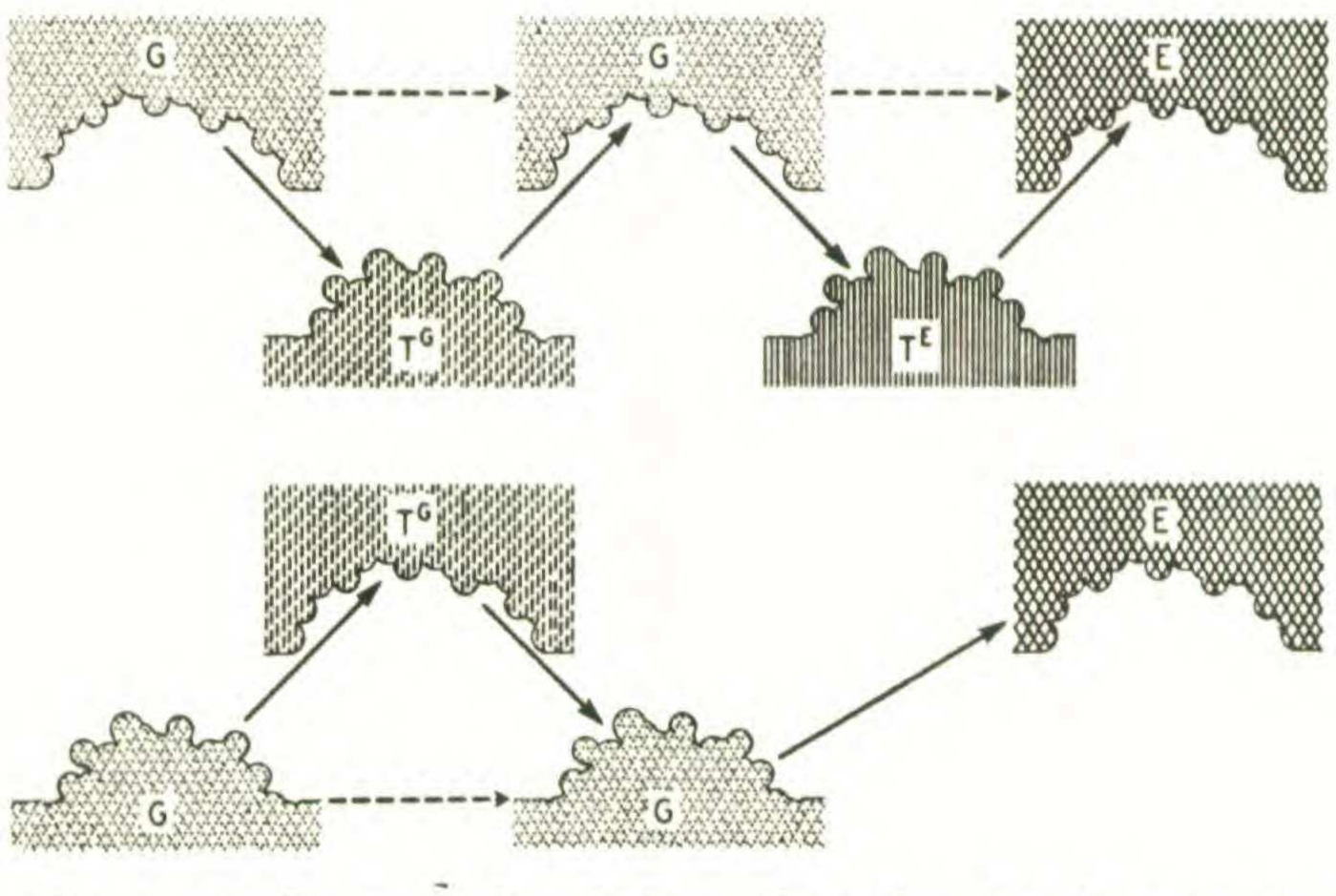

Fig. 3. Possible routes by which specific surfaces can be transmitted from gene (G) to gene and from gene to enzyme (E). T^G is a complementary gene template, T^E, an enzyme template.

that since the specificity of an antigen is genetically determined, gene and antigen may have identical surfaces, in which case antibodies developed against that antigen could combine with the corresponding face of the gene. The presence of the antibody molecule on the surface of the gene would so change that surface that the gene could no longer make an exact copy of itself, but would either fail to reproduce, or would produce a new gene with an altered surface. Either of these results would be recognizable as a mutation since the descendants of this cell could no longer elaborate the antigen in question. Experimental evidence is still inadequate on this point as there is no one case in which antibodies to a particular antigen have been shown to induce mutations in the gene responsible for the production of that antigen. If such evidence is forthcoming, it would indicate that the surface of the antigen is duplicated in either the gene itself or in the gene template.

If this scheme for the induction of mutations by antibodies should be correct, it should be possible to accomplish the same result more simply. For example, it is known that maltase activity can be inhibited by the presence of an excess of glucose. The glucose molecule fits into part of the active enzyme surface, and

when present in excess there is usually a molecule of glucose in the way of the maltose molecules which generally fit into this surface. By using very high concentrations of glucose it should be possible to have one of its molecules associated with the corresponding surface of the gene at the time of gene reproduction, resulting in mutation in the same way as when antibodies are present. We have made a few attempts along these lines by treating *Neurospora* with high concentrations of different sugars, analogues known to give substrate inhibition with certain enzymes, etc. Except for one mutation to be discussed later, the method has not proved too satisfactory, resulting usually in a great deal of sterility.

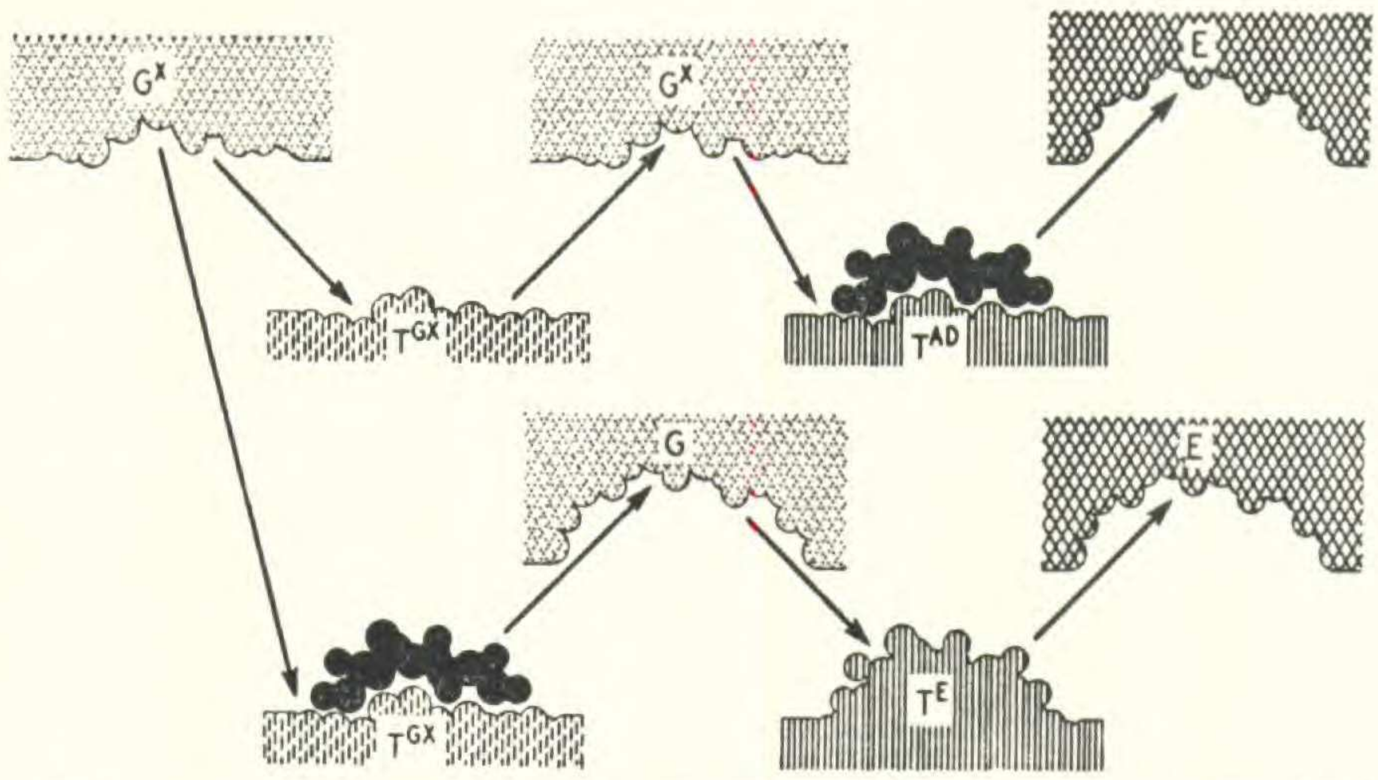

Fig. 4. Formation of adaptive enzymes: T^{AD}, adaptive template (maltose shown in in black); G^X, gene; T^{GX}, gene template; E, enzyme (maltase); G, mutated gene; T^E, enzyme (maltase) template. Further explanation in text.

The scheme just outlined can be extended to account for certain adaptive phenomena. The upper half of fig. 4 illustrates "cytoplasmic" or non-inherited adaptation. G^X represents a different gene from the one previously illustrated, and is shown as reproducing itself by means of a template (T^{GX}). The role of this gene is to produce a nucleoprotein template (T^{AD}) in the cytoplasm. In the presence of maltose, this template attaches to a molecule of maltose by its reverse side, and the surface of the template is altered in much the same way as the surface of an antigen is altered by the presence of a haptenic group. The enzyme that is synthesized on this template-plus-maltose will have a surface configuration complementary to the surface of maltose, which gives it the surface structure of the enzyme maltase (E). In this instance, maltase is an adaptive enzyme which is produced only in the presence of maltose. This is the way I had pictured the production of the killer substance in *Paramecium* as reported by Sonneborn ('43a, '43b), but from the additional evidence he has now presented (Sonneborn, '45) it is apparent that the scheme I have outlined will not account for all of his observations. Lindegren, Spiegelman and Lindegren ('44) have shown that genes are involved in producing a background for adaptive melibiose fermentation by yeast, giving a situation that fits the scheme outlined here, but to account for the addi-

tional observations just reported by Spiegelman ('45) it would be necessary to have the adaptive template (cytoplasmic factor) self-reproducing, as he has suggested.

The lower half of the same diagram (fig. 4) shows what must happen to the gene responsible for the adaptive structure under proper circumstances. If the organism is cultured in very high concentrations of maltose, enough molecules of the sugar should get into the nucleus so that some could combine with the gene-template (T^{GX}), and the gene then constructed on this template should produce maltase ever after, even in the absence of maltose.

Two examples that may represent mutations of this sort have recently turned up in our laboratory. By culturing *Neurospora* in the presence of sulfanilamide, Cushing (unpublished) was able to adapt a strain to the extent that conidia would germinate on higher concentrations of the sulfanilamide than the non-adapted strain, and, at a given concentration, mycelial growth was more rapid in the adapted strain. The adaptive characteristics were only partially maintained after a single subculture in the absence of the drug, and completely lost on outcrossing, indicating that the adaptation was not genetic. After growing the adapted strain on still higher concentrations of sulfanilamide, a heterocaryon was obtained which contained some normal nuclei and some mutated nuclei. Isolated mutant strains were extremely tolerant to sulfanilamide and in some respects made better growth in the presence of the drug than in its absence.

The second example is the one mutation obtained following treatment of *Neurospora* with a molecular solution of lactose. Lactase is an adaptive enzyme in *Neurospora* in that it is not produced in the absence of the specific substrate. The mutant differed from wild type in that it grew poorly on all carbon sources, but, in contrast to wild type, it grew just as well on lactose as on glucose, suggesting that lactase might be produced irrespective of specific substrate. Tests are now under way to determine if this is the case. The experiments to date do not distinguish between induced and spontaneous mutations.

While there is little available experimental material bearing on the mechanisms outlined, it may be useful to have them presented at this time. The scheme has many postulated steps, but each is fairly reasonable in the light of our present knowledge, and it does give a rather unified picture. There is the further point that the scheme should be amenable to experimental attack, and if the experiments are successful we have a way of learning something about genes from a different approach by making use of the methods of enzyme chemistry and immunochemistry.

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